SECRETED AND CYTOPLASMIC TUMOR ENDOTHELIAL MARKERS

This application claims the benefit of U.S. provisional applications S.N. 60/393,023, filed July2, 2002, and S.N. 60/458,964, filed April 1, 2003.

The U.S. government retains certain rights in the invention by virtue of the provisions of National Institutes of Heath grants CA57345 and CA43460, which supported this work.

TECHNICAL FIELD OF THE INVENTION

This invention is related to the area of angiogenesis and anti-angiogenesis. In particular, it relates to genes which are characteristically expressed in tumor endothelial and normal endothelial cells.

BACKGROUND OF THE INVENTION

It is now widely recognized that tumors require a blood supply for expansive growth. This recognition has stimulated a profusion of research on tumor angiogenesis, based on the idea that the vasculature in tumors represents a potential therapeutic target. However, several basic questions about tumor endothelium remain unanswered. For example, are vessels of tumors qualitatively different from normal vessels of the same tissue? What is the relationship of tumor endothelium to endothelium of healing wounds or other physiological or pathological forms of angiogenesis? The answers to these questions critically impact on the potential for new therapeutic approaches to inhibit angiogenesis in a specific manner.

There is a continuing need in the art to characterize the vasculature of tumors relative to normal vasculature so that any differences can be exploited for therapeutic and diagnostic benefits.

One technique which can be used to characterize gene expression, or more precisely gene transcription, is termed serial analysis of gene expression (SAGE). Briefly, the SAGE approach is a method for the rapid quantitative and qualitative analysis of mRNA transcripts based upon the isolation and analysis of short defined sequence tags (SAGE Tags) corresponding to expressed genes. Each Tag is a short nucleotide sequences (9-17 base pairs in length) from a defined position in the transcript. In the SAGE method, the Tags are dimerized to reduce bias inherent in

cloning or amplification reactions. (See, US Patent 5,695,937) SAGE is particularly suited to the characterization of genes associated with vasculature stimulation or inhibition because it is capable of detecting rare sequences, evaluating large numbers of sequences at one time, and to provide a basis for the identification of previously unknown genes.

SUMMARY OF THE INVENTION

Yet another aspect of the invention is a method for identification of a ligand involved in endothelial cell regulation. A test compound is contacted with a human protein selected from the group consisting of secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or

cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of HLA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1 homolog; hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16 (yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta 3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent,

regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1. Binding of a test compound to the human protein is determined. A test compound which binds to the protein is identified as a ligand involved in endothelial cell regulation.

Still another embodiment of the invention provides a method of inhibiting neoangiogenesis in a patient. A molecule comprising an antibody variable region which specifically binds to a TEM protein selected from the group consisting of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; angiopoietin 1; PDGF alpha polypeptide;

insulin-like growth factor binding protein; and IK cytokine, down-regulator of HLA II is administered to the patient. Neoangiogenesis in the patient consequently inhibited.

Yet another aspect of the invention is a method of screening for neoangiogenesis in a patient. A body fluid collected from the patient is contacted with a molecule comprising an antibody variable region which specifically binds to a TEM protein selected from the group consisting of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; angiopoietin 1; PDGF alpha polypeptide; insulin-like growth factor binding protein; and IK cytokine, down-regulator of HLA II. Detection of cross-reactive material in the body fluid with the molecule indicates neoangiogenesis in the patient.

Also provided by the present invention is a method of promoting neoangiogenesis in a patient. A TEM protein selected from the group consising of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; angiopoietin 1; PDGF alpha polypeptide; insulin-like growth factor binding protein; and IK cytokine, down-regulator of HLA II and, is administered to a patient in need of neoangiogenesis. Neoangiogenesis in the patient is consequently stimulated.

One embodiment of the invention provides a method of promoting neoangiogenesis in a patient. A nucleic acid molecule encoding a TEM protein selected from the group consising of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII,

alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; angiopoietin 1; PDGF alpha polypeptide; insulin-like growth factor binding protein; and IK cytokine, down-regulator of HLA II, is administered to a patient in need of neoangiogenesis. The TEM protein is consequently expressed and neoangiogenesis in the patient is stimulated.

Another embodiment of the invention provides a method of screening for neoangiogenesis in a patient. A TEM protein selected from the group consisting of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1

(Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; angiopoietin 1; PDGF alpha polypeptide; insulin-like growth factor binding protein; and IK cytokine, down-regulator of HLA II, is detected in a body fluid collected from the patient. Detection of the TEM protein indicates neoangiogenesis in the patient.

Another aspect of the invention is a method of screening for neoangiogenesis in a patient. A nucleic acid encoding a TEM protein selected from the group consisting of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1;

complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; angiopoietin 1; PDGF alpha polypeptide; insulin-like growth factor binding protein; and IK cytokine, down-regulator of HLA II, is detected in a body fluid collected from the patient. Detection of the TEM protein indicates neoangiogenesis in the patient.

A still further embodiment of the invention is a method to identify candidate drugs for treating tumors. Cells which express one or more TEM genes selected from the group consisting of secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa,

dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of HLA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1 homolog; hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16 (yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta 3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho

GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent, regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1, are contacted with a test compound. Expression of said one or more TEM genes is determined by hybridization of mRNA of said cells to a nucleic acid probe which is complementary to said mRNA. A test compound is identified as a candidate drug for treating tumors if it decreases expression of said one or more TEM genes. Optionally the cells are endothelial cells. Alternatively or additionally, the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more TEMs. Test compounds which increase expression can be identified as candidates for promoting wound healing.

Yet another embodiment of the invention is a method to identify candidate drugs for treating tumors. Cells which express one or more TEM proteins selected from the group consisting of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1;

complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of HLA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1 homolog; hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16 (yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta 3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-

associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent, regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1, are contacted with a test compound. The amount of said one or more TEM proteins in said cells is determined. A test compound is identified as a candidate drug for treating tumors if it decreases the amount of one or more TEM proteins in said cells. Optionally the cells are endothelial cells. Alternatively or additionally, the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more TEMs. Alternatively, a test compound which increases the amount of one or more TEM proteins in said cells is identified as a candidate drug for treating wound healing.

According to another aspect of the invention a method is provided to identify candidate drugs for treating tumors. Cells which express one or more TEM proteins selected from the group consisting of: secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV,

autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of LA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1 homolog; hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16 (yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta

3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent, regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1, are contacted with a test compound. Activity of said one or more TEM proteins in said cells is determined. A test compound is identified as a candidate drug for treating tumors if it decreases the activity of one more TEM proteins in said cells. Optionally the cells are endothelial cells. Alternatively or additionally, the cells are recombinant host cells which are transfected with an expression construct which encodes said one or more TEMs. Optionally the cells are endothelial cells. If a test compound increases the acitivity of one more TEM proteins in said cells it can be identified as a candidate drug for treating wound healing.

An additional aspect of the invention is a method to identify candidate drugs for treating patients bearing tumors. A test compound is contacted with recombinant host cells which are transfected with an expession construct which encodes one or more TEM proteins selected from the group consisting of secreted protein, acidic, cysteine-

rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of HLA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1

homolog; hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16 (yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta 3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent, regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1. Proliferation of said cells is determined. A test compound which inhibits proliferation of said cells is identified as a candidate drug for treating patients bearing tumors. A test coumpound which stimulates proliferation of said cells is identified as a candidate drug for promoting neoangiogenesis, such as for use in wound healing.

Another aspect of the invention is a method for identifying endothelial cells. One or more antibodies which bind specifically to a TEM protein selected from the group consisting of secreted protein, acidic, cysteine-rich (osteonectin); collagen, type

I, alpha 1; collagen, type IV, alpha 1; collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of HLA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1 homolog;

hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16 (yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta 3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent, regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1, is contacted with a population of cells. Cells in the population which have bound to said antibodies are detected. Cells which are bound to said antibodies are identified as endothelial cells. Optionally cells which have bound to said antibodies are isolated from cells which have not bound.

Still another aspect of the invention is a method for identifying endothelial cells. One or more nucleic acid hybridization probes which are complementary to a TEM gene nucleic acid sequence selected from the group consisting of secreted protein, acidic, cysteine-rich (osteonectin); collagen, type I, alpha 1; collagen, type IV, alpha 1;

collagen, type XVIII, alpha 1; fibronectin 1; collagen, type IV, alpha 2; Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021); collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); collagen, type VI, alpha 2; collagen, type XVIII, alpha 1; collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant); transforming growth factor, beta-induced, 68Kd; Biglycan; collagen, type VI, alpha 1; small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK); spondin 2, extracellular matrix protein; Fibromodulin; laminin, alpha 4; collagen, type IV, alpha 1; complement component 1, s subcomponent; fibulin 1; frizzled-related protein; lysyl oxidase-like 2; plasminogen activator, urokinase; natural killer cell transcript 4; microfibrillar-associated protein 2; collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive); follistatin-like 1; complement component 1, r subcomponent; Decorin; secreted protein, acidic, cysteine-rich (osteonectin); Thy-1 cell surface antigen; cysteine-rich, angiogenic inducer, 61; immunoglobulin lambda locus; hypothetical protein CAB56184; serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1; collagen, type I, alpha 1; collagen, type V, alpha 2; laminin, beta 1; DKFZP586B0621 protein; cysteine knot superfamily 1, BMP antagonist 1; hypothetical protein FLJ23053; hypothetical protein FLJ20397; matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase); insulin-like growth factor binding protein 7; collagen, type V, alpha 1; thrombospondin 2; midkine (neurite growth-promoting factor 2); DKFZP564I1922 protein; fibrillin 1 (Marfan syndrome); transforming growth factor, beta 1; serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1; galactosidase, beta 1; IK cytokine, down-regulator of HLA II; DnaJ (Hsp40) homolog, subfamily B, member 1; heat shock 70kD protein 1A; heat shock 70kD protein 1B; lectin, galactoside-binding, soluble, 1 (galectin 1); heat shock 90kD protein 1, alpha; DnaJ (Hsp40) homolog, subfamily B, member 1; tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor); heat shock 60kD protein 1 (chaperonin); heat shock 10kD protein 1 (chaperonin 10); general transcription factor II, i; heat shock 70kD protein 6 (HSP70B'); heat shock 105kD; heat shock 105kD; eukaryotic translation initiation factor 4A, isoform 2; hypothetical protein similar to mouse Fbw5; DKFZP727M231 protein; dynein, cytoplasmic, light polypeptide; hypothetical protein MGC15875; murine retrovirus integration site 1 homolog; hypothetical protein FLJ22376; smoothelin; vacuolar protein sorting 16

(yeast homolog); peanut (Drosophila)-like 2; hypothetical protein FLJ10350; FK506-binding protein 4 (59kD); proteasome (prosome, macropain) subunit, beta type, 6; transgelin; sorting nexin 17; ribosomal protein S6 kinase, 90kD, polypeptide 4; kinesin family member 1C; BTB (POZ) domain containing 2; guanylate cyclase 1, soluble, beta 3; protein-L-isoaspartate (D-aspartate) O-methyltransferase; D-aspartate oxidase; chromosome 9 open reading frame 3; regulator of G-protein signalling 16; voltage-dependent anion channel 3; NS1-binding protein; interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD); carbonic anhydrase II; protein phosphatase 2, regulatory subunit B (B56), gamma isoform; chromosome 14 open reading frame 3; eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD); Rho GTPase activating protein 1; RAP1B, member of RAS oncogene family; profilin 1; DKFZP586L151 protein; hypothetical protein FLJ14987; mitogen-activated protein kinase kinase 1 interacting protein 1; chimerin (chimaerin) 1; hephaestin; KIAA0196 gene product; melanoma-associated antigen recognised by cytotoxic T lymphocytes; HLA class II region expressed gene KE2; histamine N-methyltransferase; hypothetical protein FLJ10842; TIA1 cytotoxic granule-associated RNA-binding protein; N-acylaminoacyl-peptide hydrolase; integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2, MSK12); DKFZP586J0119 protein; hepatocyte growth factor-regulated tyrosine kinase substrate; regulator of G-protein signalling 1; proteasome (prosome, macropain) subunit, beta type, 7; KIAA1402 protein; crystallin, alpha B; protein kinase C, zeta; protein kinase, cAMP-dependent, regulatory, type II, alpha; homologous to yeast nitrogen permease (candidate tumor suppressor); intestinal cell kinase; GS3955 protein; activated p21cdc42Hs kinase; Rho-associated, oiled-coil-containing protein kinase I; KIAA2002 protein; unc-51-like kinase 1; and PDGFA associated protein 1, is contacted with nucleic acids of a population of cells. Nucleic acids which have specifically hybridized to said nucleic acid hybridization probes are detected. Cells whose nucleic acids specifically hybridized are identified as endothelial cells.

These and other embodiments which will be apparent to those of skill in the art upon reading the specification provide the art with reagents and methods for detection, diagnosis, therapy, and drug screening pertaining to neoangiogenesis and pathological processes involving or requiring neoangiogenesis.

DETAILED DESCRIPTION OF THE INVENTION

We identified 123 human genes that were expressed at significantly higher levels (> 2-fold) in tumor endothelium than in normal endothelium. See Tables 1 and 3, which show extracellular and cytoplasmic tumor endothelial markers (TEMs) respectively. Tables 2 and 4 identify the structure of the genes, proteins, and mRNAs that correspond to the tags identified. See also Tables 5, 6, and 7. Most of these genes were either not expressed or expressed at relatively low levels in Endothelial Cells (ECs) maintained in culture. Interestingly, the tumor endothelium genes were expressed in all tumors tested, regardless of its tissue or organ source. Most tumor endothelium genes were also expressed in corpus luteum and wounds.

Table 1. Extracellular tumor endothelial markers.

SEQ ID NO	StdTag	Function
1	ATGTGAAGAG	secreted protein, acidic, cysteine-rich (osteonectin)
2	ACCAAAAACC	collagen, type I, alpha 1
3	GACCGCAGGA	collagen, type IV, alpha 1
4	TAATCCTCAA	collagen, type XVIII, alpha 1
5	ATCTTGTTAC	fibronectin 1
6	TTCTCCCAAA	collagen, type IV, alpha 2
7	TCTCTGATGC	Homo sapiens mRNA; cDNA DKFZp586J021 (from clone DKFZp586J021)
8	GATCAGGCCA	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
9	GTGCTAAGCG	collagen, type VI, alpha 2
10	GACAGGCTGG	collagen, type XVIII, alpha 1
11	CGTCTTTAAA	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)
12	GTGTGTTTGT	transforming growth factor, beta-induced, 68Kd
13	GCCTGTCCCT	Biglycan
14	TTGCTGACTT	collagen, type VI, alpha 1
15	CAGGTTTCAT	small inducible cytokine subfamily B (Cys-X-Cys), member 14 (BRAK)
16	TTATGGATCT	spondin 2, extracellular matrix protein
17	AGTGGTGGCT	Fibromodulin
18	ACAGAGCACA	laminin, alpha 4
19	GTGCTACTTC	collagen, type IV, alpha 1
20	ACTGAAAGAA	complement component 1, s subcomponent
21	CAGCTGGCCA	fibulin 1
22	CTGTTACCAG	frizzled-related protein
23	TGTCATCACA	lysyl oxidase-like 2

SEQ ID NO	StdTag	Function
24	ACCTGTGACC	plasminogen activator, urokinase
25	TCCTCTTTCC	natural killer cell transcript 4
26	GACCACCTTT	microfibrillar-associated protein 2
27	GTGCTGATTC	collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)
28	CCGTGACTCT	follistatin-like 1
29	TTCTGTGCTG	complement component 1, r subcomponent
30	ACTTATTATG	Decorin
31	TTAGTGTCGT	secreted protein, acidic, cysteine-rich (osteonectin)
32	GCTGGTGCCT	Thy-1 cell surface antigen
33	AGTGTCTGTG	cysteine-rich, angiogenic inducer, 61
34	AAGGGAGCAC	immunoglobulin lambda locus
35	AATTCTGTAA	hypothetical protein CAB56184
36	GTCTGTCAGG	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1
37	CCGGGGGAGC	collagen, type I, alpha 1
38	GACGATCAAG	collagen, type V, alpha 2
39	CTTGTAACAG	laminin, beta 1
40	GGGCAGTGGC	DKFZP586B0621 protein
41	AATCTGTAAC	cysteine knot superfamily 1, BMP antagonist 1
42	GCTATCACAT	hypothetical protein FLJ23053
43	TTTGAAAATT	hypothetical protein FLJ20397
44	TAAATCCCCA	matrix metalloproteinase 9 (gelatinase B, 92kD gelatinase, 92kD type IV collagenase)
45	GATAACTACA	insulin-like growth factor binding protein 7
46	TGATTCTGTT	collagen, type V, alpha 1
47	GTCAAAATTT	thrombospondin 2
48	CCCTGCCTTG	midkine (neurite growth-promoting factor 2)
49	ACTGCTTTAC	DKFZP564I1922 protein
50	TGCAATATGC	fibrillin 1 (Marfan syndrome)
51	ATCGTGCGCT	transforming growth factor, beta 1
52	ACATAGACCG	serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium

SEQ ID NO	StdTag	Function
53	TTACCTTTTT	galactosidase, beta 1
54	GCTGTGGATA	IK cytokine, down-regulator of HLA II

Table 2. Extracellular tumor endothelial markers: identification of tags with gene sequences.

SEQ ID NO	Unigene ID	gene symbol	locuslink id	OMIMID	mRNA	Signal Seq
1	Hs.111779	SPARC	6678	182120	NM_003118	yes
2	Hs.172928	COL1A1	1277	120150	NM_000088	yes
3	Hs.119129	COL4A1	1282	120130	NM_001845	yes
4	Hs.78409	COL18A1	80781	120328	AF018081	yes
5	Hs.287820	FN1	2335	135600	NM_002026	yes
6	Hs.75617	COL4A2	1284	120090	NM_001846	
7	Hs.6441				NM_003255	yes
8	Hs.119571	COL3A1	1281	120180	NM_000090	yes
9	Hs.159263	COL6A2	1292	120240	NM_001849	yes
10	Hs.78409	COL18A1	80781	120328	AF018081	yes
11	Hs.119571	COL3A1	1281	120180	NM_000090	yes
12	Hs.118787	TGFBI	7045	601692	NM_000358	yes
13	Hs.821	BGN	633	301870	BC004244	yes
14	Hs.108885	COL6A1	1291	120220	NM_001848	yes
15	Hs.24395	SCYB14	9547	604186	AF144103	yes
16	Hs.288126	SPON2	10417	605918	NM_012445	no
17	Hs.230	FMOD	2331	600245	NM_002023	yes
18	Hs.78672	LAMA4	3910	600133	NM_002290	yes
19	Hs.119129	COL4A1	1282	120130	NM_001845	yes
20	Hs.169756	C1S	716	120580	NM_001734	yes
21	Hs.79732	FBLN1	2192	135820	NM_006485	yes
22	Hs.153684	FRZB	2487	605083	NM_001463	yes
23	Hs.83354	LOXL2	4017		NM_002318	yes
24	Hs.77274	PLAU	5328	191840	NM_002658	yes
25	Hs.943	NK4	9235	606001	NM_004221	no
26	Hs.83551	MFAP2	4237	156790	NM_002403,NM_	yes
27	Hs.1640	COL7A1	1294	120120	NM_000094	yes

SEQ ID NO	Unigene ID	gene symbol	locuslink id	OMIMID	mRNA	Signal Seq
28	Hs.296267	FSTL1	11167	605547	NM_007085	yes
29	Hs.1279	C1R	715	216950	NM_001733	yes
30	Hs.76152	DCN	1634	125255	NM_001920	yes
31	Hs.111779	SPARC	6678	182120	NM_003118	yes
32	Hs.125359	THY1	7070	188230	NM_006288	yes
33	Hs.8867	CYR61	3491	602369	NM_001554	yes
34	Hs.181125	IGLC3	3538		D01059	yes
35	Hs.241575				NM_032520	yes
36	Hs.151242	SERPING1	710	106100	NM_000062	yes
37	Hs.172928	COL1A1	1277	120150	NM_000088	yes
38	Hs.82985	COL5A2	1290	120190	NM_000393	no
39	Hs.82124	LAMB1	3912	150240	NM_002291	yes
40	Hs.157211				NM_015645	yes
41	Hs.40098	CKTSF1B1	26585	603054	NM_013372	yes
42	Hs.94037				NM_022907	yes
43	Hs.272688				NM_017802	no
44	Hs.151738	MMP9	4318	120361	NM_004994	yes
45	Hs.119206	IGFBP7	3490	602867	NM_001553	yes
46	Hs.146428	COL5A1	1289	120215	NM_000093	yes
47	Hs.108623	THBS2	7058	188061	NM_003247	yes
48	Hs.82045	MDK	4192	162096	NM_002391	yes
49	Hs.72157				AF245505	yes
50	Hs.750	FBN1	2200	134797	NM_000138	no
51	Hs.1103	TGFB1	7040	190180	NM_000660	yes
52	Hs.173594	SERPINF1	5176	172860	NM_002615	yes
53	Hs.79222	GLB1	2720	230500	NM_000404	yes
54	Hs.8024	IK	3550	600549	NM_006083	no

Table 3. Cytoplasmic tumor endothelial markers.

SEQ ID	StdTag	Function
55	AGACCA	DnaJ (Hsp40) homolog, subfamily B, member 1
56	CAGAG	heat shock 70kD protein 1A
57	AAGAG	heat shock 70kD protein 1B
58	GCCCC	lectin, galactoside-binding, soluble, 1 (galectin 1)
59	TACTAG	heat shock 90kD protein 1, alpha
60	AGAGG	DnaJ (Hsp40) homolog, subfamily B, member 1
61	GAGAG	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)
62	TACCAG	heat shock 60kD protein 1 (chaperonin)
63	TAAATA	heat shock 10kD protein 1 (chaperonin 10)
64	CCTTTC	general transcription factor II, I
65	TGAAAG	heat shock 70kD protein 6 (HSP70B')
66	TGAAAG	heat shock 105kD
67	TGAACC	heat shock 105kD
68	TCTTAA	eukaryotic translation initiation factor 4A, isoform 2
69	TCCACG	hypothetical protein similar to mouse Fbw5
70	GGGCC	DKFZP727M231 protein
71	GACTGT	dynein, cytoplasmic, light polypeptide
72	CACACC	hypothetical protein MGC15875
73	TTGTTA	murine retrovirus integration site 1 homolog
74	GAAGAA	hypothetical protein FLJ22376
75	GCCAG	Smoothelin
76	GTAGCA	vacuolar protein sorting 16 (yeast homolog)
77	CCGGC	peanut (Drosophila)-like 2
78	CCCCCT	hypothetical protein FLJ10350
79	CTTGAG	FK506-binding protein 4 (59kD)
80	GAGCG	proteasome (prosome, macropain) subunit, beta type, 6

SEQ ID	StdTag	Function
81	ACAGG	transgelin
82	GGCCA	sorting nexin 17
83	GTCACT	ribosomal protein S6 kinase, 90kD, polypeptide 4
84	AGGAT	kinesin family member 1C
85	CCCCC	BTB (POZ) domain containing 2
86	TTAATG	guanylate cyclase 1, soluble, beta 3
87	CTGTGT	protein-L-isoaspartate (D-aspartate) O-methyltransferase
88	CCTATA	D-aspartate oxidase
89	GCTGG	chromosome 9 open reading frame 3
90	CTTTGA	regulator of G-protein signalling 16
91	ACTGGT	voltage-dependent anion channel 3
92	CTGTAC	NS1-binding protein
93	ATTTAG	interferon-induced, hepatitis C-associated microtubular aggregate protein (44kD)
94	ATTTCA	carbonic anhydrase II
95	GGGGG	protein phosphatase 2, regulatory subunit B (B56), gamma isoform
96	TTTTCT	chromosome 14 open reading frame 3
97	CCTGTA	eukaryotic translation initiation factor 2, subunit 1 (alpha, 35kD
98	TATTTC	Rho GTPase activating protein 1
99	CAAGG	RAP1B, member of RAS oncogene family
100	GGCTG	profilin 1
101	TCATCT	DKFZP586L151 protein
102	TCGTAA	hypothetical protein FLJ14987
103	ATAGTA	mitogen-activated protein kinase kinase 1 interacting protein 1
104	CCTACA	chimerin (chimaerin) 1
105	TTTTAG	hephaestin
106	CAATTT	KIAA0196 gene product
107	CACCTT	melanoma-associated antigen recognised by cytotoxic T lymphocytes
108	GTGGG	HLA class II region expressed gene KE2
109	GATATA	histamine N-methyltransferase
110	TCTTAA	hypothetical protein FLJ10842

SEQ ID	StdTag	Function
111	TGAAAA	TIA1 cytotoxic granule-associated RNA-binding protein
112	AGCTGA	N-acylaminoacyl-peptide hydrolase
113	GTCCCA	integrin, beta 1 (fibronectin receptor, beta polypeptide, antigen CD29 includes MDF2,
114	TTGCG	DKFZP586J0119 protein
115	GTCTGT	hepatocyte growth factor-regulated tyrosine kinase substrate
116	TCCTTG	regulator of G-protein signalling 1
117	TGGCTA	proteasome (prosome, macropain) subunit, beta type, 7
118	TCTTCT	KIAA1402 protein
119	GTTTCA	crystallin, alpha B
120	CGCATT	protein kinase C, zeta
121	ATCGTG	protein kinase, cAMP-dependent, regulatory, type II, alpha
122	AGCTG	homologous to yeast nitrogen permease (candidate tumor suppressor)
123	CTCCAC	PDGFA associated protein 1

Table 4. Cytoplasmic tumor endothelial markers: identification of tags with gene sequences.

SEQ ID NO	Unigene ID	33gene symbol	locuslink id	OMIMID	mRNA	Signal Seq
55	Hs.82646	DNAJB1	3337	604572	NM_006145	No
56	Hs.8997	HSPA1A	3303	140550	NM_005345	No
57	Hs.274402	HSPA1B	3304	603012	NM_005346	No
58	Hs.227751	LGALS1	3956	150570	NM_002305	No
59	Hs.289088	HSPCA	3320	140571	NM_005348	No
60	Hs.82646	DNAJB1	3337	604572	NM_006145	No
61	Hs.5831	TIMP1	7076	305370	NM_003254	No
62	Hs.79037	HSPD1	3329	118190	BC010112	No
63	Hs.1197	HSPE1	3336	600141	NM_002157	No
64	Hs.278589	GTF2I	2969	601679	NM_032999	No
65	Hs.3268	HSPA6	3310	140555	NM_002155	No
66	Hs.36927	HSP105B	10808		NM_006644	No
67	Hs.36927	HSP105B	10808		NM_006644	No
68	Hs.173912	EIF4A2	1974	601102	NM_001967	No
69	Hs.82023				BC014130	No
70	Hs.168073					No
71	Hs.5120	PIN	8655	601562	NM_003746	No
72	Hs.315054				NM_032921	No
73	Hs.251385	MRVI1	10335	604673	NM_006069	No
74	Hs.29341				NM_022760	No
75	Hs.149098	SMTN	6525	602127	AF064238	No
76	Hs.302441				NM_022575	No
77	Hs.155524	PNUTL2	5414	603696	NM_004574	No
78	Hs.177596				AF218002	No
79	Hs.848	FKBP4	2288	600611	NM_002014	No
80	Hs.77060	PSMB6	5694	600307	BC000835	No
81	Hs.75777	TAGLN	6876	600818	NM_003186	No

SEQ ID NO	Unigene ID	33gene symbol	locuslink id	OMIMID	mRNA	Signal Seq
82	Hs.278569	SNX17	9784	605963	NM_014748	No
83	Hs.105584	RPS6KA4	8986	603606	NM_003942	No
84	Hs.139648	KIF1C	10749	603060	AB014606	No
85	Hs.25817				NM_017797	No
86	Hs.77890	GUCY1B3	2983	139397	NM_000857	No
87	Hs.79137	PCMT1	5110	176851	NM_005389	No
88	Hs.174441	DDO	8528	124450	NM_003649	No
89	Hs.18075				NM_032823	No
90	Hs.183601	RGS16	6004	602514	NM_002928	No
91	Hs.7381	VDAC3	7419		BC002456	No
92	Hs.197298	NS1	10625		AF205218	No
93	Hs.82316	MTAP44	10561		NM_006417	No
94	Hs.155097	CA2	760	259730	NM_000067	No
95	Hs.171734	PPP2R5C	5527	601645	NM_002719	No
96	Hs.204041				NM_012111	No
97	Hs.151777	EIF2S1	1965	603907	BC002513	No
98	Hs.138860	ARHGAP1	392	602732	BC018118	No
99	Hs.156764	RAP1B	5908	179530	NM_015646	No
100	Hs.75721	PFN1	5216	176610	NM_005022	No
101	Hs.43658				AL050137	No
102	Hs.11197				BC000628	No
103	Hs.6361				NM_021970	No
104	Hs.169965	CHN1	1123	118423	NM_001822	No
105	Hs.31720	HEPH	9843	300167	NM_014799	No
106	Hs.8294				NM_014846	No
107	Hs.279869	MAAT1	10573	604853	NM_006428	No
108	Hs.205736	HKE2	10471	605660	NM_014260	No
109	Hs.81182	HNMT	3176	605238	NM_006895	No
110	Hs.260238				NM_018238	No
111	Hs.239489	TIA1	7072	603518	NM_022173	No

SEQ ID NO	Unigene ID	33gene symbol	locuslink id	OMIMID	mRNA	Signal Seq
112	Hs.78223	APEH	327	102645	NM_001640	No
113	Hs.287797	ITGB1	3688	135630	AK024451	No
114	Hs.169474	EIF2B4	8890		NM_015636	No
115	Hs.24756	HGS	9146	604375	NM_004712	No
116	Hs.75256	RGS1	5996	600323	NM_002922	No
117	Hs.118065	PSMB7	5695	604030	NM_002799	No
118	Hs.86392				AB037823	yes
119	Hs.1940	CRYAB	1410	123590	NM_001885	No
120	Hs.78793	PRKCZ	5590	176982	NM_002744	No
121	Hs.286241	PRKAR2A	5576	176910	BC002763	No
122	Hs.169780				NM_006545	No
123	Hs.278426	PDAP1	11333		NM_014891	No

Table 5. Additional colon extracellular and cytoplasmic tumor endothelial markers

Hs.108850	intestinal cell kinase	Protein Kinase
Hs.155418	GS3955 protein	Protein Kinase
Hs.153937	activated p21cdc42Hs kinase	Protein Kinase
Hs.17820	Rho-associated, coiled-coil-containing protein kinase I	Protein Kinase
Hs.9587	KIAA2002 protein	Protein Kinase
Hs.47061	unc-51-like kinase 1	Protein Kinase
Hs.2463	angiopoietin 1	Extracellular Growth Factors & Cytokine
Hs.37040	PDGF alpha polypeptide	Extracellular Growth Factors & Cytokine
Hs.235935	insulin-like growth factor binding protein	Extracellular Growth Factors & Cytokine

Table 6: Extracellular Colon Tumor Endothelial Markers

Unigene ID	Function	OMIMID	Protein
Hs.101302	TEM36, COL12A1	120320	NP_004361
Hs.108623	thrombospondin 2	188061	NP_003238
Hs.108885	collagen, type VI, alpha 1	120220	NP_001839
Hs.1103	transforming growth factor, beta 1 (Camurati-Engelmann disease)	190180	NP_000651
Hs.111301	TEM7, matrix metalloproteinase 2 (gelatinase A, 72kD gelatinase, 72kD type IV collagenase)	120360	NP_004521
Hs.111779	secreted protein, acidic, cysteine-rich (osteonectin)	182120	NP_003109
Hs.118397	AE binding protein 1	602981	NP_001120
Hs.118787	transforming growth factor, beta-induced, 68kDa	601692	NP_000349
Hs.119129	TEM31, COL4A1 rev str;	120130	NP_001836
Hs.119206	insulin-like growth factor binding protein 7	602867	NP_001544
Hs.119571	collagen, type III, alpha 1 (Ehlers-Danlos syndrome type IV, autosomal dominant)	120180	NP_000081
Hs.1274	TEM25, BMP1; transcript variants were checked, BMP1-3 corresponds to tag, metalloproteinase, capable of inducing formation of cartilage	112264	NP_006120
Hs.1279	complement component 1, r subcomponent	216950	NP_001724
Hs.146428	collagen, type V, alpha 1	120215	NP_000084
Hs.151242	serine (or cysteine) proteinase inhibitor, clade G (C1 inhibitor), member 1, (angioedema, hereditary)	606860	NP_000053
Hs.151738	matrix metalloproteinase 9 (gelatinase B, 92kDa gelatinase, 92kDa type IV collagenase)	120361	NP_004985
Hs.153684	frizzled-related protein	605083	NP_001454
Hs.155324	TEM6, stromelysin; cleaves collagen, involved in tissue remodeling, migration; knockouts don't support tumor growth or metastasis as well as wild-type mice	185261	NP_005931
Hs.157211	C1q and tumor necrosis factor related protein 5		NP_056460
Hs.159263	collagen, type VI, alpha 2	120240	NP_001840
Hs.1640	collagen, type VII, alpha 1 (epidermolysis bullosa, dystrophic, dominant and recessive)	120120	NP_000085
Hs.169756	complement component 1, s subcomponent	120580	NP_001725
Hs.17144	short-chain dehydrogenase/reductase 1		NP_004744
Hs.172928	TEM23, COL1A1 rev str; claimed as no match	120150	NP_000079
Hs.173594	serine (or cysteine) proteinase inhibitor, clade F (alpha-2 antiplasmin, pigment epithelium derived factor), member 1	172860	NP_002606
Hs.179573	TEM10, COL1A2 involved in tissue remodeling	120160	NP_000080
Hs.272688	hypothetical protein FLJ20397		NP_060272
Hs.287820	fibronectin 1	135600	NP_002017
Hs.288126	spondin 2, extracellular matrix protein	605918	BAB15789
Hs.296267	follistatin-like 1	605547	NP_009016
Hs.4909	TEM4, DKK-3 involved in regulation of Wnt signaling pathway	605416	NP_037385
Hs.57929	TEM27, slit homolog 3 involved in cell adhesion	603745	BAA32466
Hs.5831	tissue inhibitor of metalloproteinase 1 (erythroid potentiating activity, collagenase inhibitor)	305370	NP_003245

Unigene ID	Function	OMIMID	Protein
Hs.699	peptidylprolyl isomerase B (cyclophilin B)	123841	NP_000933
Hs.202097	procollagen C-endopeptidase enhancer	600270	NP_002584
Hs.230	fibromodulin	600245	NP_002014
Hs.241575	hypothetical protein CAB56184		NP_115909
Hs.356624	TEM11, nidogen (enactin)	131390	NP_002499
Hs.375599	immunoglobulin lambda joining 3		
Hs.40098	cysteine knot superfamily 1, BMP antagonist 1	603054	NP_037504
Hs.418	fibroblast activation protein, alpha	600403	NP_004451
Hs.72157	adlican		NP_056234
Hs.750	fibrillin 1 (Marfan syndrome)	134797	NP_000129
Hs.75410	heat shock 70kDa protein 5 (glucose-regulated protein, 78kDa)	138120	NP_005338
Hs.75617	collagen, type IV, alpha 2	120090	NP_001837
Hs.76152	decorin	125255	NP_001911
Hs.77274	plasminogen activator, urokinase	191840	NP_002649
Hs.78409	collagen, type XVIII, alpha 1	120328	NP_085059
Hs.78672	laminin, alpha 4	600133	NP_002281
Hs.79222	galactosidase, beta 1	230500	NP_000395
Hs.79732	fibulin 1	135820	NP_006476
Hs.79914	TEM37, lumican; ECM proteoglycan	600616	NP_002336
Hs.80988	TEM12, COL6A3 involved in tissue remodeling	120250	NP_004360
Hs.82045	midkine (neurite growth-promoting factor 2)	162096	NP_002382
Hs.821	biglycan	301870	NP_001702
Hs.82124	laminin, beta 1	150240	NP_002282
Hs.82985	collagen, type V, alpha 2	120190	NP_000384
Hs.83354	lysyl oxidase-like 2	606663	NP_002309
Hs.83551	microfibrillar-associated protein 2	156790	NP_059453
Hs.86392	KIAA1402 protein		BAA92640
Hs.8867	cysteine-rich, angiogenic inducer, 61	602369	NP_001545
Hs.9383	cysteine-rich with EGF-like domains 1	607170	NP_056328
Hs.94037	hypothetical protein FLJ23053		NP_075058
Hs.94795	TEM41, homology to secreted protein myocilin (olfactomedin domain) submit predicted sequence		BAB84990

Table 7: Cytoplasmic Colon Tumor Endothelial Markers

Unigene ID	Function	OMIMID	Protein
Hs.10098	putative nucleolar RNA helicase		NP_061955
Hs.105584	ribosomal protein S6 kinase, 90kDa, polypeptide 4	603606	NP_003933
Hs.11197	chromosome 20 open reading frame 92		
Hs.118065	proteasome (prosome, macropain) subunit, beta type, 7	604030	
Hs.118249	ADP-ribosylation factor guanine nucleotide-exchange factor 2 (brefeldin A-inhibited)	605371	NP_006411
Hs.1197	heat shock 10kDa protein 1 (chaperonin 10)	600141	NP_002148
Hs.151777	eukaryotic translation initiation factor 2, subunit 1 alpha, 35kDa	603907	NP_004085
Hs.155097	carbonic anhydrase II	259730	NP_000058
Hs.172702	folliculin	607273	NP_659434
Hs.173912	eukaryotic translation initiation factor 4A, isoform 2	601102	NP_001958
Hs.260238	hypothetical protein FLJ10842		NP_060708
Hs.278569	sorting nexin 17	605963	NP_055563
Hs.279772	brain specific protein		NP_057224
Hs.279869	mitochondrial ribosomal protein L28	604853	
Hs.29341	chromosome 20 open reading frame 81		NP_073597
Hs.302441	vacuolar protein sorting 16 (yeast)		NP_072097
Hs.315054	hypothetical protein MGC15875		NP_699204
Hs.3268	heat shock 70kDa protein 6 (HSP70B')	140555	NP_002146
Hs.327412	TEM15, COL3A1, Homo sapiens clone FLC1492 PRO3121 mRNA, complete cds		
Hs.334790	hypothetical protein FLJ14675		NP_116212
Hs.351432	TEM24,tensin	600076	NP_072174
Hs.43658	DKFZP586L151 protein		CAB43286
Hs.5120	dynein, cytoplasmic, light polypeptide 1	601562	NP_003737
Hs.57813	zinc ribbon domain containing, 1	607525	NP_055411
Hs.6361	mitogen-activated protein kinase kinase 1 interacting protein 1	603296	
Hs.198182	TEM21, DKFZp434G162		CAB82400
Hs.227751	lectin, galactoside-binding, soluble, 1 (galectin 1)	150570	NP_002296

Unigene ID	Function	OMIMID	Protein
Hs.239752	nuclear receptor subfamily 2, group F, member 6	132880	NP_005225
Hs.353207	solute carrier family 26, member 10		NP_597996
Hs.356137	homologous to yeast nitrogen permease (candidate tumor suppressor)	607072	NP_006536
Hs.36927	heat shock 105kD		NP_006635
Hs.75721	profilin 1	176610	NP_005013
Hs.75777	transgelin	600818	NP_003177
Hs.76853	DnaJ (Hsp40) homolog, subfamily A, member 4		
Hs.77060	proteasome (prosome, macropain) subunit, beta type, 6	600307	NP_002789
Hs.77890	guanylate cyclase 1, soluble, beta 3	139397	NP_000848
Hs.79137	protein-L-isoaspartate (D-aspartate) O-methyltransferase	176851	NP_005380
Hs.81182	histamine N-methyltransferase	605238	NP_008826
Hs.82316	interferon-induced protein 44		NP_006408
Hs.82646	DnaJ (Hsp40) homolog, subfmaily B, member 1	604572	NP_006136
Hs.848	FK506 binding protein 4, 59kDa	600611	NP_002005
Hs.8997	heat shock 70kDa protein 1A	140550	NP_005336

It is clear that normal and tumor endothelium are highly related, sharing many endothelial cell specific markers. It is equally clear that the endothelium derived from tumors is qualitatively different from that derived from normal tissues of the same type and is also different from primary endothelial cultures. These genes are characteristically expressed in tumors derived from several different tissue types, documenting that tumor endothelium, in general, is different from normal endothelium. The genes expressed differentially in tumor endothelium are also expressed during other angiogenic processes such as corpus luteum formation and wound healing. It is therefore more appropriate to regard the formation of new vessels in tumors as "neoangiogenesis" rather than "tumor angiogenesis" *per se*. This distinction is important from a variety of perspectives, and is consistent with the idea that tumors recruit vasculature using much of, or basically the same signals elaborated during other physiologic or pathological processes. That tumors represent "unhealed wounds" is one of the oldest ideas in cancer biology.

Isolated and purified nucleic acids, according to the present invention are those which are not linked to those genes to which they are linked in the human genome. Moreover, they are not present in a mixture such as a library containing a multitude of distinct sequences from distinct genes. They may be, however, linked to other genes such as vector sequences or sequences of other genes to which they are not naturally adjacent. Tags disclosed herein, because of the way that they were made, represent sequences which are 3' of the 3' most restriction enzyme recognition site for the tagging enzyme used to generate the SAGE tags. In this case, the tags are 3' of the most 3' most NlaIII site in the cDNA molecules corresponding to mRNA. Nucleic acids corresponding to tags may be

RNA, cDNA, or genomic DNA, for example. Such corresponding nucleic acids can be determined by comparison to sequence databases to determine sequence identities. Sequence comparisons can be done using any available technique, such as BLAST, available from the National Library of Medicine, National Center for Biotechnology Information. Tags can also be used as hybridization probes to libraries of genomic or cDNA to identify the genes from which they derive. Thus, using sequence comparisons or cloning, or combinations of these methods, one skilled in the art can obtain full-length nucleic acid sequences. Genes corresponding to tags will contain the sequence of the tag at the 3' end of the coding sequence or of the 3' untranslated region (UTR), 3' of the 3' most recognition site in the cDNA for the restriction endonuclease which was used to make the tags. The nucleic acids may represent either the sense or the anti-sense strand. Nucleic acids and proteins althought disclosed herein with sequence particularity, may be derived from a single individual. Allelic variants which occur in the population of humans are including within the scope of such nucleic acids and proteins. Those of skill in the art are well able to identify allelic variants as being the same gene or protein. Given a nucleic acid, one of ordinary skill in the art can readily determine an open reading frame present, and consequently the sequence of a polypeptide encoded by the open reading frame and, using techniques well known in the art, express such protein in a suitable host. Proteins comprising such polypeptides can be the naturally occurring proteins, fusion proteins comprising exogenous sequences from other genes from humans or other species, epitope tagged polypeptides, etc. Isolated and purified proteins are not in a cell, and are separated from the normal cellular constituents, such as nucleic acids, lipids, etc. Typically the protein is purified to such an extent that it comprises the predominant species of protein in

the composition, such as greater than 50, 60 70, 80, 90, or even 95% of the proteins present.

Using the proteins according to the invention, one of ordinary skill in the art can readily generate antibodies which specifically bind to the proteins. Such antibodies can be monoclonal or polyclonal. They can be chimeric, humanized, or totally human. Any functional fragment or derivative of an antibody can be used including Fab, Fab', Fab2, Fab'2, and single chain variable regions. So long as the fragment or derivative retains specificity of binding for the endothelial marker protein it can be used. Antibodies can be tested for specificity of binding by comparing binding to appropriate antigen to binding to irrelevant antigen or antigen mixture under a given set of conditions. If the antibody binds to the appropriate antigen at least 2, 5, 7, and preferably 10 times more than to irrelevant antigen or antigen mixture then it is considered to be specific.

Techniques for making such partially to fully human antibodies are known in the art and any such techniques can be used. According to one particularly preferred embodiment, fully human antibody sequences are made in a transgenic mouse which has been engineered to express human heavy and light chain antibody genes. Multiple strains of such transgenic mice have been made which can produce different classes of antibodies. B cells from transgenic mice which are producing a desirable antibody can be fused to make hybridoma cell lines for continuous production of the desired antibody. See for example, Nina D. Russel, Jose R. F. Corvalan, Michael L. Gallo, C. Geoffrey Davis, Liise-Anne Pirofski. Production of Protective Human Antipneumococcal Antibodies by Transgenic Mice with Human Immunoglobulin Loci *Infection and Immunity* April 2000, p. 1820-1826; Michael L. Gallo, Vladimir E. Ivanov, Aya Jakobovits, and C. Geoffrey Davis. The human

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Antibodies can also be made using phage display techniques. Such techniques can be used to isolate an initial antibody or to generate variants with altered specificity or avidity characteristics. Single chain Fv can also be used as is convenient. They can be made from vaccinated transgenic mice, if desired. Antibodies can be produced in cell culture, in phage, or in various animals, including but not limited to cows, rabbits, goats, mice, rats, hamsters, guinea pigs, sheep, dogs, cats, monkeys, chimpanzees, apes.

Antibodies can be labeled with a detectable moiety such as a radioactive atom, a chromophore, a fluorophore, or the like. Such labeled antibodies can be used for diagnostic techniques, either *in vivo*, or in an isolated test sample. Antibodies can also be conjugated, for example, to a pharmaceutical agent, such as chemotherapeutic drug or a toxin. They can be linked to a cytokine, to a ligand, to another antibody. Suitable agents for coupling to antibodies to achieve an anti-tumor effect include cytokines, such as interleukin 2 (IL-2) and Tumor Necrosis Factor (TNF); photosensitizers, for use in photodynamic therapy, including aluminum (III) phthalocyanine tetrasulfonate, hematoporphyrin, and phthalocyanine; radionuclides, such as iodine-131 (^{131}I), yttrium-90 (^{90}Y), bismuth-212 (^{212}Bi), bismuth-213 (^{213}Bi), technetium-99m (^{99m}Tc), rhenium-186 (^{186}Re), and rhenium-188 (^{188}Re); antibiotics, such as doxorubicin, adriamycin, daunorubicin, methotrexate, daunomycin, neocarzinostatin, and carboplatin; bacterial,

plant, and other toxins, such as diphtheria toxin, pseudomonas exotoxin A, staphylococcal enterotoxin A, abrin-A toxin, ricin A (deglycosylated ricin A and native ricin A), TGF-alpha toxin, cytotoxin from chinese cobra (naja naja atra), and gelonin (a plant toxin); ribosome inactivating proteins from plants, bacteria and fungi, such as restrictocin (a ribosome inactivating protein produced by *Aspergillus restrictus*), saporin (a ribosome inactivating protein from *Saponaria officinalis*), and RNase; tyrosine kinase inhibitors; ly207702 (a difluorinated purine nucleoside); liposomes containing antitumor agents (*e.g.*, antisense oligonucleotides, plasmids which encode for toxins, methotrexate, etc.); and other antibodies or antibody fragments, such as F(ab).

Those of skill in the art will readily understand and be able to make such antibody derivatives, as they are well known in the art. The antibodies may be cytotoxic on their own, or they may be used to deliver cytotoxic agents to particular locations in the body. The antibodies can be administered to individuals in need thereof as a form of passive immunization.

Characterization of extracellular regions for cell surface and secreted proteins from the protein sequence can be based on the prediction of signal sequence, transmembrane domains and functional domains. Antibodies are preferably specifically immunoreactive with membrane associated proteins, particularly to extracellular domains of such proteins or to secreted proteins. Such targets are readily accessible to antibodies, which typically do not have access to the interior of cells or nuclei. However, in some applications, antibodies directed to intracellular proteins may be useful as well. Moreover, for diagnostic purposes, an intracellular protein may be an equally good target since cell lysates may be used rather than a whole cell assay.

Computer programs can be used to identify extracellular domains of proteins whose sequences are known. Such programs include SMART software (Schultz et al., Proc. Natl. Acad. Sci. USA *95:* 5857-5864, 1998) and Pfam software (Bateman et al., Nucleic acids Res. *28:* 263-266, 2000) as well as PSORTII. Typically such programs identify transmembrane domains; the extracellular domains are identified as immediately adjacent to the transmembrane domains. Prediction of extracellular regions and the signal cleavage sites are only approximate. It may have a margin of error + or – 5 residues. Signal sequence can be predicted using three different methods (Nielsen et al, *Protein Engineering* 10: 1-6 ,1997, Jagla et. al, Bioinformatics 16: 245-250 , 2000, Nakai, K and Horton, P. Trends in Biochem. Sci. 24:34-35, 1999) for greater accuracy. Similarly transmembrane (TM) domains can be identified by multiple prediction methods. (Pasquier, et. al, Protein Eng. 12:381-385, 1999, Sonnhammer et al., In Proc. of Sixth Int. Conf. on Intelligent Systems for Molecular Biology, p. 175-182 , Ed J. Glasgow, T. Littlejohn, F. Major, R. Lathrop, D. Sankoff, and C. Sensen Menlo Park, CA: AAAI Press, 1998 , Klein, et.al, Biochim. Biophys. Acta, 815:468, 1985, Nakai and Kanehisa Genomics, 14: 897-911 , 1992). In ambiguous cases, locations of functional domains in well characterized proteins are used as a guide to assign a cellular localization.

Putative functions or functional domains of novel proteins can be inferred from homologous regions in the database identified by BLAST searches (Altschul et. al. Nucleic Acid Res. 25: 3389-3402, 1997) and/or from a conserved domain database such as Pfam (Bateman et.al, Nucleic Acids Res. 27:260-262 1999) BLOCKS (Henikoff, et. al, Nucl. Acids Res. 28:228-230, 2000) and SMART (Ponting, et. al, Nucleic Acid Res. 27,229-232, 1999). Extracellular domains include regions adjacent to a transmembrane domain in

a single transmembrane domain protein (out–in or type I class). For multiple transmembrane domains proteins, the extracellular domain also includes those regions between two adjacent transmembrane domains (in-out and out-in). For type II transmembrane domain proteins, for which the N-terminal region is cytoplasmic, regions following the transmembrane domain is generally extracellular. Secreted proteins on the other hand do not have a transmembrane domain and hence the whole protein is considered as extracellular.

Membrane associated proteins can be engineered to delete the transmembrane domains, thus leaving the extracellular portions which can bind to ligands. Such soluble forms of transmembrane receptor proteins can be used to compete with natural forms for binding to ligand. Thus such soluble forms act as inhibitors. and can be used therapeutically as anti-angiogenic agents, as diagnostic tools for the quantification of natural ligands, and in assays for the identification of small molecules which modulate or mimic the activity of a TEM:ligand complex.

Alternatively, the endothelial markers themselves can be used as vaccines to raise an immune response in the vaccinated animal or human. For such uses, a protein, or immunogenic fragment of such protein, corresponding to the intracellular, extracellular or secreted TEM of interest is administered to a subject. The immogenic agent may be provided as a purified preparation or in an appropriately expressing cell. The administration may be direct, by the delivery of the immunogenic agent to the subject, or indirect, through the delivery of a nucleic acid encoding the immunogenic agent under conditions resulting in the expression of the immunogenic agent of interest in the subject. The TEM of interest may be delivered in an expressing cell, such as a purified population

of tumor endothelial cells or a populations of fused tumor endothelial and dendritic cells. Nucleic acids encoding the TEM of interest may be delivered in a viral or non-viral delivery vector or vehicle. Non-human sequences encoding the human TEM of interest or other mammalian homolog can be used to induce the desired immunologic response in a human subject. For several of the TEMs of the present invention, mouse, rat or other ortholog sequences are described herein or can be obtained from the literature or using techniques well within the skill of the art.

Endothelial cells can be identified using the markers which are disclosed herein as being endothelial cell specific. These include the human markers identified by SEQ ID NOS: 1-123, *i.e.*, the normal, pan-endothelial, and the tumor endothelial markers. Antibodies specific for such markers can be used to identify such cells, by contacting the antibodies with a population of cells containing some endothelial cells. The presence of cross-reactive material with the antibodies identifies particular cells as endothelial. Similarly, lysates of cells can be tested for the presence of cross-reactive material. Any known format or technique for detecting cross-reactive material can be used including, immunoblots, radioimmunoassay, ELISA, immunoprecipitation, and immunohistochemistry. In addition, nucleic acid probes for these markers can also be used to identify endothelial cells. Any hybridization technique known in the art including Northern blotting, RT-PCR, microarray hybridization, and in situ hybridization can be used.

One can identify tumor endothelial cells for diagnostic purposes, testing cells suspected of containing one or more TEMs. One can test both tissues and bodily fluids of a subject. For example, one can test a patient's blood for evidence of intracellular and

membrane associated TEMs, as well as for secreted TEMs. Intracellular and/or membrane associated TEMs may be present in bodily fluids as the result of high levels of expression of these factors and/or through lysis of cells expressing the TEMs.

Populations of various types of endothelial cells can also be made using the antibodies to endothelial markers of the invention. The antibodies can be used to purify cell populations according to any technique known in the art, including but not limited to fluorescence activated cell sorting. Such techniques permit the isolation of populations which are at least 50, 60, 70, 80, 90, 92, 94, 95, 96, 97, 98, and even 99 % the type of endothelial cell desired, whether normal, tumor, or pan-endothelial. Antibodies can be used to both positively select and negatively select such populations. Preferably at least 1, 5, 10, 15, 20, or 25 of the appropriate markers are expressed by the endothelial cell population.

Populations of endothelial cells made as described herein, can be used for screening drugs to identify those suitable for inhibiting the growth of tumors by virtue of inhibiting the growth of the tumor vasculature.

Populations of endothelial cells made as described herein, can be used for screening candidate drugs to identify those suitable for modulating angiogenesis, such as for inhibiting the growth of tumors by virtue of inhibiting the growth of endothelial cells, such as inhibiting the growth of the tumor or other undesired vasculature, or alternatively, to promote the growth of endothelial cells and thus stimulate the growth of new or additional large vessel or microvasculature.

Inhibiting the growth of endothelial cells means either regression of vasculature which is already present, or the slowing or the absence of the development of new

vascularization in a treated system as compared with a control system. By stimulating the growth of endothelial cells, one can influence development of new (neovascularization) or additional vasculature development (revascularization). A variety of model screen systems are available in which to test the angiogenic and/or anti-angiogenic properties of a given candidate drug. Typical tests involve assays measuring the endothelial cell response, such as proliferation, migration, differentiation and/or intracellular interaction of a given candidate drug. By such tests, one can study the signals and effects of the test stimuli. Some common screens involve measurement of the inhibition of heparanase, endothelial tube formation on Matrigel, scratch induced motility of endothelial cells, platelet-derived growth factor driven proliferation of vascular smooth muscle cells, and the rat aortic ring assay (which provides an advantage of capillary formation rather than just one cell type).

Drugs can be screened for the ability to mimic or modulate, inhibit or stimulate, growth of tumor endothelium cells and/or normal endothelial cells. Drugs can be screened for the ability to inhibit tumor endothelium growth but not normal endothelium growth or survival. Similarly, human cell populations, such as normal endothelium populations or tumor endothelial cell populations, can be contacted with test substances and the expression of tumor endothelial markers and/or normal endothelial markers determined. Test substances which decrease the expression of tumor endothelial markers (TEMs) are candidates for inhibiting angiogenesis and the growth of tumors. In cases where the activity of a TEM is known, agents can be screened for their ability to decrease or increase the activity.

For those tumor endothelial markers identified as being secreted proteins, it is desirable to identify drug candidates capable of binding to the secreted TEM protein. For

some applications, the identification of drug candidates capable of interfering with the binding of the secreted TEM to its native receptor is desirable. For other applications, the identification of drug candidates capable of mimicking the activity of the native receptor will be desired. Thus, by manipulating the binding of the secreted TEM:receptor complex, one may be able to promote or inhibit futher development of endothelial cells, and hence, vascularization.

Expression can be monitored according to any convenient method. Protein or mRNA can be monitored. Any technique known in the art for monitoring specific genes' expression can be used, including but not limited to ELISAs, SAGE, microarray hybridization, Western blots. Changes in expression of a single marker may be used as a criterion for significant effect as a potential pro-angiogenic, anti-angiogenic or anti-tumor agent. However, it also may be desirable to screen for test substances which are able to modulate the expression of at least 5, 10, 15, or 20 of the relevant markers, such as the tumor or normal endothelial markers. Inhibition of TEM protein activity can also be used as a drug screen. Human and mouse TEMS can be used for this purpose.

Test substances for screening can come from any source. They can be libraries of natural products, combinatorial chemical libraries, biological products made by recombinant libraries, etc. The source of the test substances is not critical to the invention. The present invention provides means for screening compounds and compositions which may previously have been overlooked in other screening schemes. Nucleic acids and the corresponding encoded proteins of the markers of the present invention can be used therapeutically in a variety of modes. TEMs can be used to stimulate the growth of vasculature, such as for wound healing or to circumvent a blocked vessel. The nucleic

acids and encoded proteins can be administered by any means known in the art. Such methods include, using liposomes, nanospheres, viral vectors, non-viral vectors comprising polycations, etc. Suitable viral vectors include adenovirus, retroviruses, and sindbis virus. Administration modes can be any known in the art, including parenteral, intravenous, intramuscular, intraperitoneal, topical, intranasal, intrarectal, intrabronchial, etc.

Specific biological antagonists of TEMs can also be used to therapeutic benefit. For example, antibodies, T cells specific for a TEM, antisense to a TEM, and ribozymes specific for a TEM can be used to restrict, inhibit, reduce, and/or diminish tumor or other abnormal or undesirable vasculature growth. Such antagonists can be administered as is known in the art for these classes of antagonists generally. Anti-angiogenic drugs and agents can be used to inhibit tumor growth, as well as to treat diabetic retinopathy, rheumatoid arthritis, psoriasis, polycystic kidney disease (PKD), and other diseases requiring angiogenesis for their pathologies.

The above disclosure generally describes the present invention. All references disclosed herein are expressly incorporated by reference. A more complete understanding can be obtained by reference to the following specific examples which are provided herein for purposes of illustration only, and are not intended to limit the scope of the invention.

EXAMPLE 1

Visualization of vasculature of colorectal cancers

The endothelium of human colorectal cancer was chosen to address the issues of tumor angiogenesis, based on the high incidence, relatively slow growth, and resistance to

anti-neoplastic agents of these cancers. While certain less common tumor types, such as glioblastomas, are highly vascularized and are regarded as good targets for anti-angiogenic therapy, the importance of angiogenesis for the growth of human colorectal cancers and other common solid tumor types is less well documented.

We began by staining vessels in colorectal cancers using von Willebrand Factor (vWF) as a marker. In each of 6 colorectal tumors, this examination revealed a high density of vessels throughout the tumor parenchyma (Examples in Fig. 1 A and B). Interestingly, these analyses also substantiated the importance of these vessels for tumor growth, as endothelium was often surrounded by a perivascular cuff of viable cells, with a ring of necrotic cells evident at the periphery (Example in Fig. 1A). Although these preliminary studies suggested that colon tumors are angiogenesis-dependent, reliable markers that could distinguish vessels in colon cancers from the vessels in normal colon are currently lacking. One way to determine if such markers exist is by analyzing gene expression profiles in endothelium derived from normal and neoplastic tissue.

EXAMPLE 2

Purification of endothelial cells

Global systematic analysis of gene expression in tumor and normal endothelium has been hampered by at least three experimental obstacles. First, endothelium is enmeshed in a complex tissue consisting of vessel wall components, stromal cells, and neoplastic cells, requiring highly selective means of purifying ECs for analysis. Second, techniques for defining global gene expression profiles were not available until recently. And third, only

a small fraction of the cells within a tumor are endothelial, mandating the development of methods that are suitable for the analysis of global expression profiles from relatively few cells.

To overcome the first obstacle, we initially attempted to purify ECs from dispersed human colorectal tissue using CD31, an endothelial marker commonly used for this purpose. This resulted in a substantial enrichment of ECs but also resulted in contamination of the preparations by hematopoietic cells, most likely due to expression of CD31 by macrophages. We therefore developed a new method for purifying ECs from human tissues using P1H12, a recently described marker for ECs. Unlike CD31, P1H12 was specifically expressed on the ECs of both colorectal tumors and normal colorectal mucosa. Moreover, immunofluorescence staining of normal and cancerous colon with a panel of known cell surface endothelial markers (e.g. VE-cadherin, CD31 and CD34) revealed that P1H12 was unique in that it stained all vessels including microvessels (see Fig. 2A and data not shown). In addition to selection with P1H12, it was necessary to optimize the detachment of ECs from their neighbors without destroying their cell surface proteins as well as to employ positive and negative affinity purifications using a cocktail of antibodies (Fig. 2B). The ECs purified from normal colorectal mucosa and colorectal cancers were essentially free of epithelial and hematopoietic cells as judged by RT-PCR (Fig. 2C) and subsequent gene expression analysis (see below).

EXAMPLE 3

Comparison of tumor and normal endothelial cell expression patterns

To overcome the remaining obstacles, a modification of the Serial Analysis of Gene Expression (SAGE) technique was used. SAGE associates individual mRNA transcripts with 14 base pair tags derived from a specific position near their 3' termini. The abundance of each tag provides a quantitative measure of the transcript level present within the mRNA population studied. SAGE is not dependent on pre-existing databases of expressed genes, and therefore provides an unbiased view of gene expression profiles. This feature is particularly important in the analysis of cells that constitute only a small fraction of the tissue under study, as transcripts from these cells are unlikely to be well represented in extant EST databases. We adapted the SAGE protocol so that it could be used on small numbers of purified ECs obtained from the procedure outlined in Fig. 2B.

A library of ~100,000 tags from the purified ECs of a colorectal cancer, and a similar library from the ECs of normal colonic mucosa from the same patient were generated. These ~193,000 tags corresponded to over 32,500 unique transcripts. Examination of the expression pattern of hematopoietic, epithelial and endothelial markers confirmed the purity of the preparations (Fig. 2D).

EXAMPLE 7

Tumor endothelium markers are neo-angiogenic

Finally, we asked whether these transcripts were expressed in angiogenic states other than that associated with tumorigenesis. We thus performed in situ hybridizations on corpus luteum tissue as well as healing wounds. Although there were exceptions, we found

that these transcripts were generally expressed both in the corpus luteum and in the granulation tissue of healing wounds. In all tissues studied, expression of the genes was either absent or exclusively confined to the EC compartment.

References and Notes

The disclosure of each reference cited is expressly incorporated herein.

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8. The original EC isolation protocol was the same as that shown in Fig. 2B except that dispersed cells were stained with anti-CD31 antibodies instead of anti-P1H12, and magnetic beads against CD64 and CD14 were not included in the negative selection. After generating 120,000 SAGE tags from these two EC preparations, careful analysis of the SAGE data revealed that, in addition to endothelial-specific markers, several macrophage-specific markers were also present.

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11. In order to reduce the minimum amount of starting material required from ~50 million cells to ~50,000 cells (i.e. ~1000-fold less) we and others (38) have introduced

several modifications to the original SAGE protocol. A detailed version of our modified "MicroSAGE" protocol is available from the authors upon request.

12. 96,694 and 96,588 SAGE tags were analyzed from normal and tumor derived ECs, respectively, and represented 50,298 unique tags. A conservative estimate of 32,703 unique transcripts was derived by considering only those tags observed more than once in the current data set or in the 134,000 transcripts previously identified in human transcriptomes (39).

13. To identify endothelial specific transcripts, we normalized the number of tags analyzed in each group to 100,000, and limited our analysis to transcripts that were expressed at levels at least 20-fold higher in ECs than in non-endothelial cell lines in culture and present at fewer than 5 copies per 100,000 transcripts in non-endothelial cell lines and the hematopoietic fraction (~57,000 tags)(41). Non-endothelial cell lines consisted of 1.8x106 tags derived from a total of 14 different cancer cell lines including colon, breast, lung, and pancreatic cancers, as well as one non-transformed keratinocyte cell line, two kidney epithelial cell lines, and normal monocytes. A complete list of PEMs is available at www.sagenet.org\angio\table1.htm.

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26. For non-radioactive in situ hybridization, digoxigenin (DIG)-labelled sense and anti-sense riboprobes were generated through PCR by amplifying 500-600 bp products and incorporating a T7 promoter into the anti-sense primer. In vitro transcription was performed using DIG RNA labelling reagents and T7 RNA polymerase (Roche, Indianapolis, IN). Frozen tissue sections were fixed with 4 % paraformaldehyde, permeabilized with pepsin, and incubated with 200 ng/ml of riboprobe overnight at 55oC. For signal amplification, a horseradish peroxidase (HRP) rabbit anti-DIG antibody (DAKO, Carpinteria, CA) was used to catalyse the deposition of Biotin-Tyramide (from GenPoint kit, DAKO). Further amplification was achieved by adding HRP rabbit anti-biotin (DAKO), biotin-tyramide, and then alkaline-phosphatase (AP) rabbit anti-biotin (DAKO). Signal was detected using the AP substrate Fast Red TR/Napthol AS-MX (Sigma, St. Louis, MO), and cells were counterstained with hematoxylin unless otherwise indicated. A detailed protocol including the list of primers used to generate the probes can be obtained from the authors upon request.

27. Transcript copies per cell were calculated assuming an average cell contains 300,000 transcripts.

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31. Endothelial-specific transcripts were defined as those expressed at levels at least 5-fold higher in ECs in vivo than in non-endothelial cell lines in culture (13), and present at no more than 5 copies per 100,000 transcripts in non-endothelial cell lines and the hematopoietic cell fraction (41). Transcripts showing statistically different levels of expression ($P < 0.05$) were then identified using Monte Carlo analysis as previously described (40). Transcripts preferentially expressed in normal endothelium were then defined as those expressed at levels at least 10-fold higher in normal endothelium than in tumor endothelium. Conversely, tumor endothelial transcripts were at least 10-fold higher in tumor versus normal endothelium. See www.sagenet.org\angio\table2.htm and www.sagenet.org\angio\table3.htm for a complete list of differentially expressed genes.

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41. Human colon tissues were obtained within ½ hour after surgical removal from patients. Sheets of epithelial cells were peeled away from normal tissues with a glass slide following treatment with 5 mM DDT, then 10 mM EDTA, leaving the lamina propria intact. After a 2h incubation in collagenase at 37 oC, cells were filtered sequentially through 400 um, 100 um, 50 um and 25 um mesh, and spun through a 30 % pre-formed Percoll gradient to pellet RBCs. Epithelial cells (Epithelial Fraction), which were found to non-specifically bind magnetic beads, were removed using Dynabeads coupled to BerEP4 (Dynal, Lake Success, NY). Subsequently, macrophages and other leukocytes (Hematopoietic Fraction) were removed using a cocktail of beads coupled to anti-CD45, anti-CD14 and anti-CD64 (Dynal). The remaining cells were stained with P1H12 antibody, purified with anti-mouse IgG-coupled magnetic beads, and lysed in mRNA lysis buffer. A detailed protocol can be obtained from the authors upon request.

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